

## Original Article

# Impaired endothelial function in adolescents with overweight or obesity measured by peripheral artery tonometry

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**Background:** Overweight and obesity in adolescents are associated with a subsequent increased mortality due to cardiovascular disease in adulthood. The reactive hyperemia-peripheral artery tonometry (RH-PAT) is a non-invasive method for endothelial function assessment.

**Objective:** The goal of this study is to investigate endothelial function as assessed with the RH-PAT in adolescents with overweight or obesity.

**Methods:** In 27 adolescents with overweight or obesity (16 males, 11 females) and 25 control subjects (12 males, 13 females) (age 12–20 yr) RH-PAT score and baseline pulse amplitude were measured after an overnight fast.

Confounding risk factors for endothelial dysfunction, including smoking and diabetes mellitus were excluded.

**Results:** RH-PAT score was lower in adolescents with overweight or obesity compared to healthy controls, whereas their baseline pulse amplitude was higher ( $p = 0.027$  and  $p < 0.0001$ , respectively). A significantly positive correlation was seen between baseline pulse amplitude and body mass index standard deviation score in the group with overweight or obese subjects.

**Conclusions:** Endothelial dysfunction, measured by lower RH-PAT score and higher baseline pulse amplitude, was present in overweight adolescents.

Interestingly, we also report for the first time in the literature a significant difference in baseline pulse amplitude between overweight adolescents compared to their peers.

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**Key words:** adolescents – obesity – overweight – PAT – vascular endothelium

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Childhood overweight and obesity have become major public health issues. According to the International Obesity Taskforce, the percentage of overweight children in Belgium reaches 10–15%, and obesity 2% (<http://www.who.org/childhoodobesity.asp>). Overweight in childhood is associated with an increased mortality due to cardiovascular disease in adulthood, independent of adult weight (1). In the study by Franks et al., a positive association between childhood obesity and the risk of premature death, defined as death

before 55 yr of age, was shown. Children in the highest quartile of age-standardized and sex-standardized body mass index (BMI) had significantly higher rates of premature death than did children in the lowest quartile (2). Endothelial dysfunction is the suggested link between pediatric patients with overweight or obesity and the development of premature cardiovascular disease (CVD). Indeed, in adult studies, it has been shown that endothelial dysfunction precedes atherosclerotic plaque formation. Early detection of this process

may have therapeutic and prognostic implications (3). Endothelial dysfunction is described as the inability of the artery to sufficiently dilate in response to an appropriate endothelial stimulus. Endothelial function is a subclinical measure for evaluating early vascular changes in patients with increased future cardiovascular risk. Assessment of digital hyperemia is gaining prominence as a non-invasive tool to test endothelial function and has been evaluated in the pediatric population (4–8). Using reactive hyperemia-peripheral artery tonometry (RH-PAT), we evaluated endothelial function in a young adolescent group with overweight or obesity compared to a normal weight control group. The goal of this study was to determine whether a significant difference in RH-PAT score or baseline pulse amplitude was found in adolescents with overweight or obesity as compared to controls.

## Methods

### Study participants

Thirty-one adolescents with overweight (17 males and 14 females) and 27 healthy age- and sex-matched control subjects (13 males and 14 females) were recruited from our outpatient clinic between January 2009 and March 2013. All patients who participated in this study were Caucasian and aged 12–20 yr. Overweight and obesity were defined by using the age-adjusted BMI cutoff points suggested by Cole et al. in children under the age of 18 (9). At the age of 18 yr, the adult cutoff points were used ( $\text{BMI} \geq 25 \text{ kg/m}^2$  for overweight and  $\text{BMI} \geq 30 \text{ kg/m}^2$  for obesity). All subjects were non-smokers. Controls were excluded when there was a familial history of premature CVD (having first- or second-degree relatives with premature CVD). Two children from the control group were excluded from the study, resulting in 25 control subjects (12 males and 13 females). Exclusion criteria for the overweight subjects included a familial history of premature CVD or a personal history of overweight, but not longer reaching the overweight criteria on the testing day thanks to lifestyle changes. Four patients needed to be excluded resulting in 27 overweight subjects (16 males and 11 females).

### Study design

Overweight and obese patients from the outpatient clinic at the University Hospitals Leuven, and their parents, received verbal and written information before providing written consent. The control group was already recruited in our previous study and consisted of friends or family of patients with type 1 diabetes (8). The study was approved by the Ethical Review Board of the University Hospitals.

The study was registered as NCT01267591. Reactive hyperemia-peripheral artery tonometry (RH-PAT) endothelial function was assessed using the Endo-PAT device (Itamar Medical Ltd., Caesara, Israel). All measurements were performed in the same noise- and temperature-controlled room, in fasting state in the morning. The patient was placed in a comfortable position with the hands at heart level with the fingers hanging freely to avoid muscular activity. They were instructed to remain quiet during the measurements. Two plethysmographic registration probes were placed on both index fingers. After 5 min of baseline measurement, arterial flow to the non-dominant arm was occluded for 5 min using a blood pressure cuff inflated to 40 mmHg above systolic pressure. After the 5-min occlusion, the cuff deflated rapidly to allow for reactive or flow-mediated hyperemia. Pulse wave amplitudes were recorded for at least 5 min after the cuff is deflated. Owing to the concurrent signal from the contralateral finger, the method corrects for changes in systemic vascular tone. The probes were connected to a computer for online registration of all recordings. A specialized software program calculated the ratio of the average blood volume before and after occlusion in both fingers to know the RH-PAT score (10). The RH-PAT score was calculated as the ratio of the average pulse wave amplitude during the 1-min period starting 60 s after cuff deflation divided by the average pulse wave amplitude of a 210-s preocclusion baseline period. Lower RH-PAT scores reflect greater endothelial dysfunction. Following RH-PAT testing, height (without shoes), weight (in underwear, without shoes), blood pressure (BP), and Tanner score were recorded and blood was obtained in fasting condition for lipid profile, blood glucose, insulin, and hemoglobin A1c (HbA1c). Insulin resistance (IR) or a decreased sensitivity or responsiveness to metabolic actions of insulin was measured by the homeostasis model assessment (HOMA), fasting glucose/insulin ratio (FGIR), and quantitative insulin sensitivity check index (QUICKI). The HOMA index was calculated as the product of fasting glucose concentration (in mmol/L) times fasting insulin concentration (in mU/L) divided by 22.5 (11). Increases in HOMA and insulin values and decreases in FGIR reflect insulin resistance. QUICKI was derived by calculating the inverse of the sum of logarithmically expressed fasting glucose (in mg/dL) and insulin concentrations (in mU/L) (12). As insulin concentrations increase, QUICKI values decrease.

### Statistical methods

All data are presented by the median and interquartile range. The primary endpoints of the study were the RH-PAT score and the baseline pulse amplitude.

Other variables were analyzed as secondary endpoints. The characteristics, RH-PAT values and baseline pulse amplitudes in overweight cases were compared to controls (unpaired analysis, Mann–Whitney *U* test). Correlations (rank) between RH-PAT and BMI standard deviation score (SDS) were studied and between baseline pulse amplitude and BMI SDS. A *p* value below 0.05 was considered to be statistically significant. The statistical analysis was performed with the program MEDCALC<sup>®</sup> version 11.4.3 (Mariakerke, Ghent, Belgium).

## Results

### Baseline characteristics

We refer to Table 1 for an overview of the baseline characteristics of the study patients. No difference was observed in variables like age, fasting glucose levels, or Tanner score. As expected, the group with overweight or obesity individuals had significantly higher BMI values ( $p < 0.0001$ ) compared to controls. The lipid profile was more atherogenic in the subjects with overweight having statistically significantly higher triglycerides [72.0 mg/dL (64.0–121.0) vs. 64.0 mg/dL (43.8–81.8),  $p = 0.0164$ ], a significantly higher low-density lipoprotein (LDL) level [92.0 mg/dL (76.3–102.8) vs. 76.0 mg/dL (64.5–89.3),  $p = 0.006$ ] and a significantly lower high-density lipoprotein (HDL) level [45.0 mg/dL (41.3–49.8) vs. 58.0 mg/dL (49.8–63.8),  $p = 0.0012$ ]. Systolic blood pressure differed significantly between the two groups with a statistically significant higher systolic BP SDS [1.6 (1.0–2.2) vs. 0.9 (0.08–1.5),  $p = 0.007$ ] in overweight and obese adolescents. Overweight or obese adolescents had higher insulin values compared to controls, but the results were not significant.

### RH-PAT and baseline pulse amplitude

Children with overweight or obesity ( $n = 27$ ) had overt endothelial dysfunction as evidenced by lower median RH-PAT scores vs. controls [1.51 (1.27–1.90) vs. 1.88 (1.67–2.42),  $p = 0.027$ ] and higher baseline pulse amplitude [416.30 (360.3–675.7) vs. 145.29 (52.3–300.2),  $p < 0.0001$ ]. The data of this analysis are shown in Fig. 1.

### Analysis of RH-PAT and baseline pulse amplitude as continuous variables

Continuous analysis of RH-PAT scores in function of different continuous variables showed a significantly positive correlation with age [ $r = 0.4$  (0.03–0.68),  $p = 0.035$ ] and Tanner score [ $r = 0.45$  (0.05–0.73),  $p = 0.03$ ]. A significant negative correlation was

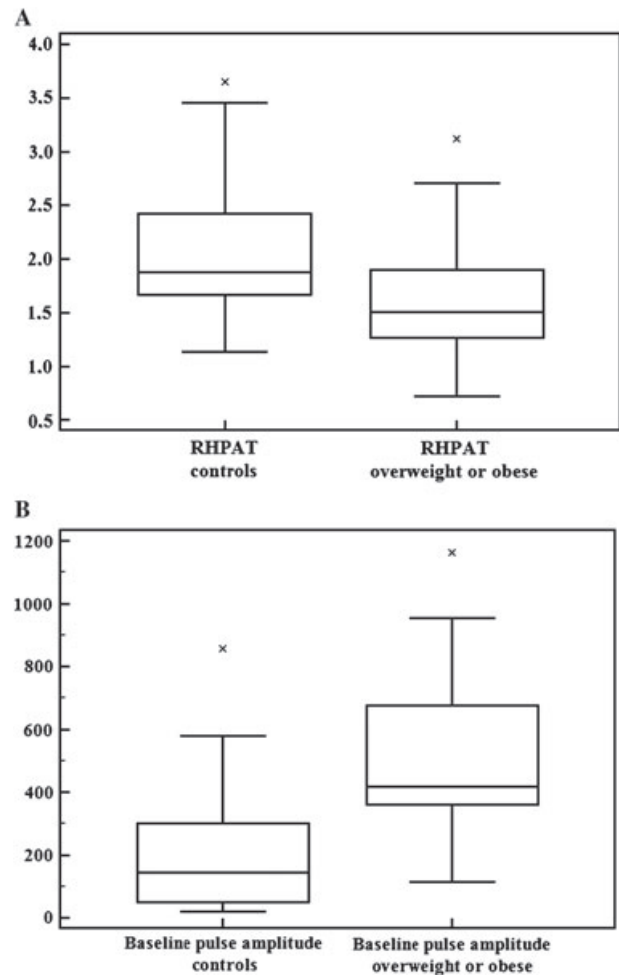


Fig. 1. Box plot of reactive hyperemia-peripheral artery tonometry (RH-PAT) score in controls vs. subjects with overweight or obesity,  $p = 0.027$  (A) and baseline pulse amplitude in controls vs. subjects with overweight or obesity,  $p < 0.0001$  (B).

seen between RH-PAT and diastolic BP SDS [ $r = -0.5$  (–0.74 to –0.15),  $p = 0.008$ ]. The correlation between RH-PAT and systolic BP SDS was nearly significant [ $r = -0.38$  (–0.66 to –0.0005),  $p = 0.05$ ]. No correlation was found between RH-PAT and the other variables, such as BMI SDS, glucose, insulin, HbA1c, total cholesterol, LDL, HDL, or triglycerides.

A significantly positive correlation was found between baseline pulse amplitude and BMI SDS [ $r = 0.39$  (0.006–0.67),  $p = 0.047$ ] in the group with overweight or obese subjects (Fig. 2). The other variables (age, Tanner score, diastolic or systolic BP, glucose, insulin, HbA1c, total cholesterol, LDL, HDL, or triglycerides) were not statistically significant.

## Discussion

In this cross-sectional study, we measured endothelial function by RH-PAT score and baseline pulse amplitude. Adolescents with overweight or obesity

Table 1. Demographic and biochemical characteristics of the study patients

Characteristics	Controls (n = 25)	Overweight (n = 27)	p value
Male gender (n)	12	16	—
Age (yr)	15.5 (13.9–16.2)	14.7 (13.0–16.4)	0.61
BMI SDS	0 [–0.3 to 0.5]	2.6 (2.0–3.0)	<0.0001
Obesity/n	—	16/27	—
Tanner	4 (3–5)	5 (3–5)	0.65
Systolic BP SDS	0.9 (0.08–1.5)	1.6 (1.0–2.2)	0.007
Diastolic BP SDS	0.1 (–0.4 to 0.5)	0.2 (–0.2 to 1.0)	0.73
Glucose (mg/dL)	92.0 (85.0–98.5)	91.0 (82.3–94.0)	0.39
Insulin (mU/L)	10.4 (7.7–14.8)	14.6 (9.1–24.3)	0.07
FGIR	8.5 (6.3–11)	6.4 (3.8–10.1)	0.06
QUICKI	0.34 (0.32–0.35)	0.32 (0.30–0.34)	0.12
HOMA	2.28 (1.68–3.59)	3.39 (2.03–5.60)	0.10
HbA1c (%)	5.3 (5.2–5.4)	5.4 (5.2–5.5)	0.16
Total cholesterol (mg/dL)	146.0 (130.0–164.0)	159.0 (136.0–166.5)	0.21
LDL (mg/dL)	76.0 (64.5–89.3)	92.0 (76.3–102.8)	0.006
HDL (mg/dL)	58.0 (49.8–63.8)	45.0 (41.3–49.8)	0.0012
Triglycerides (mg/dL)	64.0 (43.8–81.8)	72.0 (64.0–121.0)	0.0164

BMI, body mass index; BP, blood pressure; FGIR, fasting glucose/insulin ratio; HDL, high-density lipoprotein; HbA1c, Hemoglobin A1c; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; QUICKI, quantitative insulin sensitivity check index; SDS, standard deviation score. All values reported as median (P25–P75).

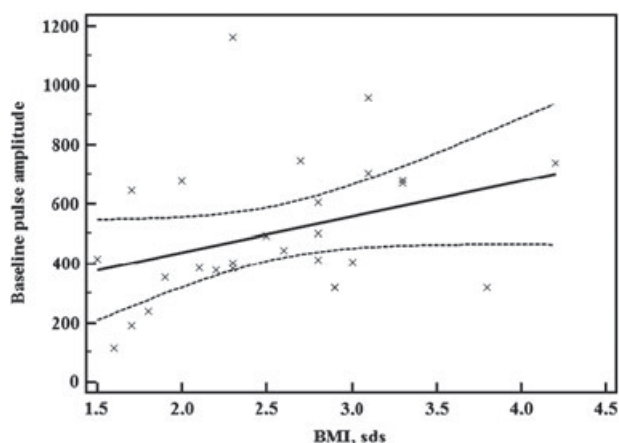


Fig. 2. Significantly positive correlation between baseline pulse amplitude and BMI SDS in the group with overweight or obese subjects,  $r = 0.39$  (0.006–0.67),  $p = 0.047$ .

had a significantly lower RH-PAT score as compared to controls, whereas their baseline pulse amplitude was higher. RH-PAT uses fingertip plethysmography probes to measure the changes in pulse wave amplitude observed before and after the period of reactive hyperemia (4). Endothelium-mediated changes in vascular tone after occlusion of the brachial artery are reflecting a downstream hyperemic response, which is a measure for arterial endothelial function. An unequivocal theory about the underlying mechanism of the RH-PAT is lacking. It is known that the provoked endothelium-dependent flow-mediated vasodilatation after removing the occlusion is partly (60%) mediated by endothelial release of nitric oxide (NO) (13). In adults, the Framingham cohort study described a

direct relation between several cardiovascular risk factors such as tobacco use, cholesterol, BMI, diabetes mellitus, and baseline pulse amplitude and an inversely association with RH-PAT score (7). Previous studies of early vascular changes in the pediatric obese population have reported inconsistent findings. Only two previous studies have used the RH-PAT score to demonstrate alterations in endothelial function in adolescents with overweight. Our results are consistent with the data from the study of Mahmud et al. They showed a significantly lower RH-PAT score in obese adolescents with insulin resistance in relation to healthy controls, indicative of impaired endothelial function (14). In contrast, in the study of Tryggstad et al., the RH-PAT score did not differ between the normal weight and overweight group (15). In the control group, the RH-PAT score was positively correlated with age. This relationship was not evident in the overweight group although the RH-PAT score tended to be lower in obese children at the older end of the age range studied. The results of this study suggested that the RH-PAT score might begin to decline in overweight adolescents after the age of 15 yr as there was a trend in that direction. This might explain the discordance with our data, as our cohort was older than the cohort in the study of Tryggstad et al. (14.7 vs. 13.9 yr). If this hypothesis could be confirmed, it would suggest that exposure to the effects of obesity for several years might be required before microvascular reactive hyperemia is adversely affected. This would be consistent with the Framingham cohort study which describes an inverse relation between the RH-PAT score and BMI in adults (7). In the study of Mahmud



et al., however, the overweight group had already lower RH-PAT scores compared to controls at a mean age of 13.4 yr old. This could be explained by the higher absolute BMI in this group compared to our group (34.8 vs. 29.5 kg/m<sup>2</sup>) and the negative association between BMI and the RH-PAT score, as clearly described by Mahmud et al. (14). Further research on the relation between age and RH-PAT score in overweight subjects would be interesting. Additionally, we observed higher baseline pulse amplitude in obese and overweight individuals compared to those of normal weight and a significantly positive correlation between baseline pulse amplitude and BMI SDS. To our knowledge, differences in baseline pulse amplitude in obese adolescents have not been previously reported. Our group, however, described similar findings in adolescents with type 1 diabetes, with significantly higher baseline pulse amplitude in the diabetes group vs. the control group (8). These findings were consistent with the data from the study of Hamburg et al. in adults (7). They observed that baseline pulse amplitude was directly related to several cardiovascular risk factors, including hypertension, diabetes mellitus, overweight, and smoking. Baseline pulse amplitude was highly dependent on digital blood flow and sympathetic tone, as evidenced by a marked reduction in digital pulse amplitude after the administration of phenylephrine, an  $\alpha$ -adrenergic vasoconstrictor agent (13). Decreased digital microvessel tone, increased blood flow, and altered microvascular structure might all contribute to this higher resting pulse amplitude. Our findings were consistent with the study of Mitchell et al. that showed an association between obesity in adults and a higher resting blood flow in the brachial artery (16). The presence of increased basal blood flow represents hyperperfusion, which might contribute to microvascular hypertension and microvessel damage in obese individuals.

IR might be a proposed link between obesity and vascular stiffness (17). In our study, insulin levels were increased in the overweight group, although not significantly. This could be due to the relatively small sample size that limits the power of the study. Hemodynamic actions of insulin include both vasodilator and vasoconstrictor effects. Insulin stimulates the production of NO in vascular endothelium, while IR may impair insulin-stimulated production of NO. Furthermore, insulin also stimulates the release of endothelin that opposes vasodilator effects of NO. Thus, the net vasoactive effect of insulin may depend on the balance between NO-dependent and NO-independent actions elicited. As a result, IR is accompanied by selective impairment of insulin action in the vasculature. Consequently, compensatory hyperinsulinemia that serves to maintain glucose homeostasis also favors

prohypertensive effects of insulin (17). In our study, BP, especially systolic BP, was higher in the group of overweight and obese adolescents. This might also have an important effect on the distal microcirculation.

Some limitations of the study are acknowledged. First, our study has a relatively small sample size that limits the power of the study. Second, we do not specifically analyze visceral adiposity, known as a predictor of cardiovascular risk in adults. Finally, the RH-PAT technique also has its limitations. An unequivocal theory about the underlying mechanism is lacking. There is some uncertainty about the relative importance of endothelium-dependent and endothelium-independent vasodilatation in the RH-PAT response. The vascular bed (and thus RH-PAT) is highly responsive to sympathetic tone and thus to external influences, such as anger, depression, and anxiety (18). There is also a high cost associated with the finger probes as each probe can only be used once.

In conclusion, overweight and obesity are globally increasing health problems for young people. Using RH-PAT, we evaluated endothelial function in this risk group compared to a normal weight control group. Our study showed a significantly lower RH-PAT score in adolescents with overweight or obesity as compared to controls, whereas their baseline pulse amplitude was higher. Moreover, a significantly positive correlation was found between baseline pulse amplitude and BMI SDS. Considering that the endothelial dysfunction is a reversible process, further research is required about therapeutic life style effects on RH-PAT score and baseline pulse amplitude.

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